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The Association Between Periodontal Disease and C-Reactive Protein In Patients With a History Of Heart Attack

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ASSOCIATION BETWEEN PERIODONTAL DISEASE AND C-REACTIVE

PROTEIN IN PATIENTS WITH A HISTORY OF HEART ATTACK

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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PROTEIN IN PATIENTS WITH A HISTORY OF HEART ATTACK

By Robert Lee Fletcher III, B.S., D.M.D.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

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The patient population consisted of a maximum of 18,570 subjects who completed the NHANES III questionnaire and examination from 1988 - 1994. The physical examination included such things as body mass index and serum samples, social and medical history. The periodontal examination recorded probing depth, attachment loss and gingival bleeding. Serum samples were analyzed for CRP levels, cholesterol levels etc. Demographic, cardiovascular and oral health variables were compared in subjects with a history of heart attack. Result showed that history of heart attack is associated with increased odds ratio for elevated CRP, diabetes, hypertension, cholesterol, male gender, non-white race and smoking. Of the periodontal indicators of disease, only gingival bleeding had an increased odds ratio for association with heart attack history. The unadjusted odds ratio was 1.25 with 95% CI[0.84-1.87]. The

adjusted odds ratio increase to 1.93 with 95% CI [1.02-3.71]. These findings are

consistent with previous research indicating that elevated CRP is associated with

increased risk of heart attack. The interesting finding of this study is that only gingival

bleeding, not probing depth or attachment loss, had an increased odds ratio for an

associated with self-reported history of heart attack.

Introduction

Periodontal disease is a chronic, low-grade bacterial infection involving the

supporting structures of the teeth. The disease severity is classified according to the

amount of attachment loss that has occurred. This loss of bone, connective tissue and

periodontal ligament (PDL) occurs during an inflammatory reaction, triggered by bacteria

and mediated by the host. Recent studies into the acute phase response and its

involvement in the process of atherosclerosis have shed new light on the possible link

with periodontal disease.[1,2,3,4,5,6]

Löe and Theilade[7] showed that inflammation of the gingiva is triggered by

bacteria, and gingival health returns once the bacteria are removed. They described the

qualitative changes in the gingival soft tissues during the progression from gingival

health to gingivitis. Page and Schroeder[8] reported that periodontal disease has a host

inflammatory component. They described the host mediated inflammatory reaction to the

bacterial trigger. Types and ratios of host cells defined the initial, early, established and

advanced lesions. Histological analysis showed that the host response consisted of

localized inflammation with infiltration of host immune cells, alteration of soft tissue

structure, and progressive breakdown of the supporting structures of the teeth.

Numerous studies have shown that bacteria and bacterial products are capable of

direct tissue invasion.[9,10,11,12] Oral microflora, and the cascade of events that they trigger,

have both local and systemic ramifications. Putative periodontal bacteria have been

found within arterial plaques.[13] Host immune cells specific for oral bacteria have been shown to cause widespread systemic complications. Virulence factors of oral bacteria contribute to the pathogenesis of gingivitis and periodontitis.[14,15,16] Once these bacteria and their products infiltrate the soft tissue, they interact with the host defenses, which consists of the cellular and humoral immune response.

The cellular immune response refers to the localized reactions to foreign material. The cells involved in this response are the lymphocytes, natural killer cells and phagocytes. These immune cells recognize the antigen, bind it and facilitate chemical breakdown of the antigen. During this process, parts of the antigen are displayed on the outer surface of these cells, which then triggers activation of the humoral immune response. T-cells are responsible for the cell-mediated immunity and are characterized by specific surface antigen receptors. When activated, T-cells proliferate and differentiate into memory T-cells and the various types of regulatory and effector T-cells such as cytotoxic, helper, and suppressor cells. Macrophages function in phagocytosis of microorganisms, digestion and presentation of antigens to T and B-lymphocytes, and secretion of various products, including enzymes, complement components, and regulatory molecules (interferon, IL-1).[17] Guo et al.,[18] studied the T-cell response in periodontal disease. Results showed that CD4+ and CD8+ lymphocytes expressed T1- and T2- type cytokine messages. T1-type cytokine messages are the major activator for cytotoxic T-cell differentiation, while the T2-type cytokine message is the major activator for Helper T-cells. This shows that the immune response to periodontal disease triggers both cytotoxic and helper T-cells.

The humoral immune response involves antibody, complement and other soluble molecules. Chemical messengers, called cytokines, activate this branch of the immune system. Inflamed tissues, which have been compromised by the invading bacteria, also release cytokines. Many cells, including the cells of the cellular immune response, produce these cytokines. B-cells are responsible for the humoral immunity and are the precursors of antibody producing B-cells, also known as plasma cells. B-cells are characterized by the presence of surface immunoglobulin. When stimulated by antigen, a process that requires the cooperation of helper T-cells and macrophages, B-cells proliferate and differentiate into plasma cells and memory cells.[19] Mackler et al.,[20] studied the different subclasses of immunoglobulin (antibody) formed in response to putative periodontal pathogens.

Activation of the host immune response involves recruitment of immune cells and production of protective antibodies.[21] Chronic stimulation of the immune response due to constant assault by oral bacteria on the periodontium causes release of proinflammatory cytokines and other proteins which likely promote soft tissue and bone destruction.[21] Zappa et al.,[22] found that there were significantly more fibroblasts, mast cells, monocytes/macrophages, and inflammatory cells for progressing periodontitis sites compared to the non-progressing sites. Seymour et al.,[23] explained the shift in immune cell types within a progressing periodontal lesion. They described the shift from a predominantly T-cell lesion to a B-cell (plasma cell) lesion. Th1 cells release interleukin (IL-2) and interferon-gamma (IFN-gamma), while Th2 cells release IL-4, IL-5, IL-6, and IL-10. Th1 cells tend to activate macrophages, and also respond well to macrophage expression of antigen. Th2 cells tend to increase production of eosinophils and mast

cells, and enhance production of antibody, including IgE; these cells respond well to antigens presented by B cells. Once established, each of these responses is able to suppress the other through cytokine messengers.

The localized immune response and inflammatory reaction caused by the invading oral flora during periodontal disease triggers a systemic response from the host. As we stated earlier, oral microflora, and the cascade of events that they trigger, have both local and systemic ramifications. Putative periodontal bacteria have been found within arterial plaques.[24] Host immune cells specific for oral bacteria have been shown to cause widespread systemic complications. Cytokines (IL-1, TNF, IFG, IL-6, etc) produced and released during this immune response are responsible for activating the acute-phase response (APR). Cytokines and their associated inflammatory responses perform numerous biologic activities. Many of these are problematic and therefore suggest they have a critical role in causing the destruction of bone and connective tissue in periodontitis. Serum levels and types of T-cell lymphocytes are influenced by the presence or absence of periodontal disease. Takeichi et al.,[25] showed that the characteristics of the periodontal lesion changed depending on the type of T-cell activated. Fujihashi et al.,[26] studied cytokine types found in periodontal lesions. He demonstrated that high levels of IL-6 were produced by gingival mononuclear cells but not by peripheral blood mononuclear cells unless cells were stimulated with T cell mitogen. Salvi et al.,[27] examined which T-helper cell type predominated in periodontal disease. He concluded that the monocytic (IL-1β and PGE-2) and Th1 (lL-2 and IFN-gamma) inflammatory mediator levels quantitatively dominate over the Th2 mediators (IL-4 and IL-6).

The acute-phase response (APR), unlike the humoral response, is non-specific.[17] Hepatic synthesis of acute-phase proteins (APP) is activated by numerous cytokines. In response to these inflammatory cytokines, acute-phase proteins are produced in great quantities. Some of these APP include the following: C-reactive protein (CRP), serum amyloid A protein, alpha-glycoprotein, ceruloplasmin, alpha-macroglobulins, complement components, alpha-antitrypsin, alpha-antichymotrypsin, fibrinogen, prothrombin and factor VIII.[28] C-reactive protein is clinically the most important APR protein because it serves as an indicator of disease and can be easily measured in a laboratory.[29] The APR mediates inflammation by activating complement. Complement activation allows clearance of antigens, killing of bacteria and regulation of inflammation. CRP activates complement and thus participates in sustaining inflammation.[17] It was named for its ability to interact with the C-polysaccharide of pneumococci and was the first acute phase protein described. CRP is an active part of the acute phase response (APR). Induction of the APR occurs due to infection, trauma, inflammatory processes and some malignant diseases. Numerous cytokines induce these acute phase protein changes, notably IL-1, IL-6 and TNF.[17] Biosynthesis of CRP and other APP occurs in the liver.[30] The APR is responsible for increased production of APP, fever and release of ACTH. The result of the APR is rapid acceleration of the host immune reaction. Normal levels of CRP in the serum are less than 10mcg/l, but it can reach levels of >100mg/l within 48 hours of initiation of the APR. CRP level is not influence by anemia or plasma protein changes. It has a half-life of 5-7 hours and reaches peak levels within 48-72 hours. CRP levels will return to normal levels within several days, following resolution of the inflammation or trauma.[31] Biosynthesis of these

acute phase proteins takes place in the liver and is regulated by a factor known as hepatocyte stimulating factor (HSP) – now known as IL-6.[19]   Fujihashi et al.,[26] demonstrated that high levels of IL-6 were produced by gingival mononuclear cells during periodontal disease. Many now consider quantitative CRP measurements the procedure of choice to detect and monitor acute inflammation and acute tissue destruction.[32]

Cardiovascular disease is characterized by arteriosclerosis, a generic term for three patterns of vascular disease that involve thickening and loss of elasticity of arterial walls. The primary disease is atherosclerosis, which is the formation of intimal fibrous plaques that many times have a central lipid core and which protrude into the lumen. The disease begins early in life but symptoms are not evident until later when arterial lesions precipitate organ injury. Myocardial infarction, cerebral infarction and aortic aneurysms are the major consequences of this disease.[33] Morphology of an atheromatous plaque (atheroma) consists of a fibrous cap (proliferating smooth muscle cells, macrophages, lymphocytes, foam cells etc), necrotic core (cellular debris, extracellular lipid with cholesterol crystals, and foamy macrophages) and neovascularization (proliferation of small blood vessels for nutrient supply). An atheroma is termed complicated once changes occur such as calcification, focal rupture or gross ulceration, hemorrhage and thrombosis. Thrombosis of the plaque is the most critical complication due to the possibility of partial or complete lumen blockage. Localized and systemic inflammation plays a critical role in the formation and maturation of atheromatous plaques.[33]

Much research has been focused on the association between periodontal disease and cardiovascular disease (CVD). Many risk factors are similar between CVD and

periodontal disease. Grossi et al.,[34] discussed several risk factors such as sex, age, smoking, diabetes and body mass index. Beck et al.,[35] studied the relationship of periodontal disease to carotid artery intima-media wall thickness. He found that compared to healthy subjects, those with moderate periodontitis had an odds ratio of 1.40 for thickened vessel wall (atherosclerosis), and those with severe periodontitis had an odds ratio of 2.09. Arbes et al.,[36] studied the association between extent of periodontal attachment loss and self-reported history of heart attack. The odds ratio for a heart attack increased with the severity of periodontal disease, from 1.4 for mild periodontitis up to 3.8 for severe periodontitis. Approximately half of all deaths in the U.S. can be attributed to complications of atherosclerosis. Of this number, only half of those deaths were associated with high cholesterol. The medical community needed another marker for increased risk of cardiovascular disease. CRP is a marker for inflammation and can accurately predict risk of future cardiac events.[32] The AAP parameters of care[37] state that periodontal pathogens may contribute to atherogenic changes and thromboembolic events in the coronary arteries and other arteries.

CRP functions within the acute phase response. Its precise role in the atherosclerotic event is unclear. Braunwald et al.,[29] states that CRP may activate complement and therefore may act to sustain inflammation. Strandberg et al.,[1] studied CRP level, cardiovascular risk factors and mortality in the elderly. After controlling for age and sex, baseline CRP level significantly predicted the 10-year total mortality and cardiovascular mortality. Ridker et al.,[2] studied CRP and the risk of future cardiovascular events among healthy women. He found the higher the level of CRP, the higher the risk of CVD (5 fold increase) and MI or stroke (7 fold increase). Rifai et al.,[3] looked at novel

risk factors for systemic atherosclerosis. He found that baseline plasma levels of Tc:HDL-c ratio and CRP were the strongest independent predictors of development of peripheral arterial disease. Noack et al.,[4] reported the range for CRP as a risk factor for CVD, peripheral vascular disease, or stroke is 1.34mg/dL to 6.45mg/dL and the mean of this range is 3mg/dL. These studies show that CRP is a reliable marker for cardiovascular disease risk and the risk of CVD increases as the level of CRP increases.

Elevation in systemic CRP levels can be seen in subjects with periodontal disease.[4,5,6] As the severity of periodontal disease increases, so does the level of CRP. Noack et al.,[4] studied the contribution of periodontal infection to elevated systemic CRP level. Subjects with periodontal disease had higher levels of CRP vs. healthy controls. Subjects with high clinical attachment loss had higher mean CRP levels vs. healthy controls. The presence of periodontal pathogens in samples was positively associated with elevated CRP levels. Loos et al.,[5] studied the elevation of systemic markers related to cardiovascular disease in the peripheral blood of periodontitis patients. He found that both generalized periodontitis and localized periodontitis subjects had elevated CRP vs. controls. 52% of generalized and 36% of localized periodontitis subjects were seropositive for IL-6 (a potent activator of CRP) vs 26% of controls. Ebersole et al.,[6] studied the systemic acute-phase reactants, CRP and haptoglobin, in adult periodontitis subjects. The mean CRP levels in adult periodontitis subjects were 9.12mg/dL vs 2.17mg/dL for healthy controls. An interesting finding of this study was that administration of an anti-inflammatory drug (flurbiprofen) decreased the level of CRP by 35-40%.

In order to study the association between periodontal disease and CRP we must first understand the limitations of classifying a site in the mouth as either being active or inactive. Periodontal disease has been characterized as a disease in which there are exacerbations and remissions.[38] Periodontal disease is classified according to the amount of attachment loss present, not evidence of current disease. Clinical findings for indicators of periodontal disease must be considered collectively in order to label a site as active or inactive. Clinical findings include the following: color (redness), swelling, bleeding on probing, probing depth and clinical attachment level.

Redness is associated with increased vascularity, capillaries and blood cell infiltrate. Löe and Theilade[7] included redness as a component of the gingival index. Redness was said to be a visual clue to the histologic change caused by the inflammatory process. It can be used to assess the severity of gingival inflammation. The presence of redness indicates tissues are no longer healthy. Redness alone is not a good predictor of disease progression. The absence of redness is an excellent negative predictor of disease progression.

Suppuration refers to purulent exudates or pus associated with the sulcus of a tooth. The world workshop(WWS), Annals of Periodontics,[39] defined suppuration as a neutrophil-rich variant of gingival crevicular fluid (GCF). Suppuration is said to be an uncommon finding with gingivitis alone. As periodontitis severity increases, suppuration accumulates in the pocket. In this review of literature, suppuration was found in only 3-5% of sites with periodontitis, but its presence had a strong association with progression of disease. Absence of suppuration had a high negative predictor value for progression of periodontitis.

Bleeding on probing (BOP) can be used to assess the extent of gingival inflammation.[39] Assessing BOP is complicated by factors such as the method of probing, time factor to observed bleeding and the qualitative assessment of bleeding. A meta-analysis from the Annals of periodontics[39] found that BOP was a reliable sign of inflammation and BOP is an important risk predictor for increased loss of attachment. In the analysis, the odds ratio of increased loss of attachment for patients with frequent BOP during maintenance period was 2.79. Davenport et al.,[40] studied histometric comparison of active and inactive lesions in advanced periodontitis. The study consisted of examining bleeding/suppurating and nonbleeding/nonsuppurating periodontitis lesions. The percent of infiltrated CT and the number of plasma cells and leukocytes were higher in bleeding than in nonbleeding sites. Bleeding and/or suppuration were indicative of histopathologic changes in lesions of advanced inflammatory periodontal disease but PD and AL were similar for all lesions. Caton et al.,[41] studied the association between bleeding and visual signs of interdental gingival inflammation. Bleeding was found in 39% of visually non-inflamed areas. This indicates that bleeding indices were more sensitive to inflammation than visual verification. Greensteen et al.,[42] reviewed the literature to study the role of BOP in the diagnosis of periodontal disease. The authors state that bleeding reflected the histological (or inflammatory changes), clinical and bacteriological (spirochetes and motile rods) alterations associated with periodontal disease. The authors found that BOP was better than visual signs of inflammation (color changes) as an earlier sign of gingivitis. Muhlemann et al.,[43] concluded that bleeding from the sulcus is the earliest clinical symptom of gingivitis and that it precedes discoloration and swelling of gingival units. Bleeding from the gently probed sulcus

precedes the appearance of gingival color changes and is the leading and first clinical symptom of marginal gingivitis. In a study of nearly 5000 subjects who were questioned about their oral health, and cardiovascular disease, Buhlin et al.,[44] found that bleeding was the only significant factor for increased odds ratio for self reported heart attack.

Probing depth is defined by the 1996 World Workshop of Periodontics[45] as the distance from the gingival margin to the base of the probable crevice. The periodontal pocket is important because it is the major habitat for putative periodontal pathogens and the route of invasion of bacteria and bacterial products. Kepic et al.,[46] showed that if the pocket can be cleaned such that all the plaque and most of the calculus is removed, gingival health could be returned. Increasing depths of the periodontal pocket complicate cleaning efforts. Caffessee et al.,[47] showed the difficulty in adequately cleaning deep pockets (>5mm). Long term maintenance studies have shown that in general, patients can maintain gingival health if their probing depths are not greater than 3mm. This is not to say that all pockets deeper than 3mm are "active" and will progress with continued attachment loss. Only a small percentage of longitudinally monitored sites with deep probing depths are at an increased risk for progression. The significance of probing depth must be considered. Goodson et al.,[48] showed that when left untreated, only 5.7% of deep probing depths ($\geq$4mm) lost attachment over 13 months. Lindhe et al.,[49] found that deep probing depths (> or = 5mm) have low positive predictive value for future disease progression, but have a high negative predictive value for disease progression. When probing depths are monitored over time and can be compared, increasing probing depth has predictive value. Badersten et al.,[50] found that probing depth increases of $\geq$ 1mm was 80% predictive of future attachment loss. Claffey et al.,[51] found the

combination of increased probing depth and BOP had the highest predictive value(87%), for future loss of attachment.

The aim of this study is to use the NHANES III database to collectively compare the indicators of periodontal disease to known cardiovascular risk factors in patients with a history of heart attack.

Materials and Methods

The National Health and Nutrition Examination Survey (NHANES) III involved

data collection from 1988 through 1994. Approximately 40,000 subjects, 2 months of

age and older, were randomly selected from households across the U.S. to participate.

Participants were asked to volunteer for interviews, extensive physical and dental

examinations and biochemical tests. All tests were performed in the mobile examination

center (MEC).

Serum Collection. During the MEC examination, a 6-cm^3 specimen of venous

blood was collected in a vacutainer tube containing a Ficoll heavy-density layer overlaid

with a thixotropic gel followed by ACD anticoagulant from examinees 12 years of age

and over. The serum will be analyzed for CRP levels using non-hs-CRP ELISA.

Periodontal Examination. The NHANES III periodontal examination was

performed on subject 13 years old or older. Only fully erupted permanent teeth were

measured. The exam used the NIDR probe which was yellow color-coded and graduated

at 2,4,6,8,10 and 12 millimeters. The NIDR probe point diameter was 0.38 millimeters.

The instructions given to the examiners were the following: Probe is to be held with a

light grasp not to exceed 25 grams and pointed toward the apex of the tooth. Each

measurement is rounded to the lowest whole millimeter. The probe is inserted from the

buccal aspect to measure both buccal and mesial sites. For the interproximal site the

examiner should keep the probe parallel to the long axis of the tooth even if the adjacent tooth is missing. For upper and lower molars the buccal assessment are made at the midpoint of the mesial root, keeping the probe parallel to the long axis of the tooth.

Gingival Assessment. The NHANES III gingival assessment instructions to the examiners was as follows: The buccal and mesiobuccal sites of each tooth are to be assessed. Score of 0=No bleeding, 1=bleeding, Y=cannot be assessed. Buccal aspects of each quadrant are to be carefully dried prior to gingival assessment. Begin assessment for the most posterior tooth. Placement of the probe: NIDR probe inserted no more than 2 mm into the sulcus, starting just distal to the midpoint of the buccal surface and then moved gently into the mesial interproximal area. After all sites from the facial or buccal aspect of a single quadrant are examined in this fashion, the bleeding points are scored.

Periodontal Destruction Assessment. The NHANES III periodontal destruction assessment included both an assessment of loss of attachment and furcation involvement. For loss of attachment, the exam will look at two sites per tooth, buccal and mesiobuccal. Loss of Attachment was defined as the distance in millimeters (mm) from the cementoenamel junction (CEJ) to the bottom of the pocket. At each site measured, first the distance from the free gingival margin (FGM) to the CEJ, and second the distance from the FGM to the bottom of the pocket will be measured. Where the gingival margin is subject to recession and the CEJ is exposed, the distance from the CEJ to the gingival margin is called a negative value. Measurements range from 0 to 12 millimeters.

Statistical analysis: Statistical analysis was performed using SPSS software. A bivariate analysis was performed for all variables to determine independent significance. A logistic regression model adjusted for age, gender, race, CRP level, diabetes,

hypertension, cholesterol, marital status, education, gingival bleeding and smoking was

then performed for those significant variables.

Results

Description of subjects:

Table 1 shows that a total of 18,570 patients filled out a questionnaire, which asked the following demographic information: Age, Gender, Race/ethnicity, Income, Marital status and Education.

Table 1. Demographic Characteristics of study population

Variable	N	%
Age (years)	18570	100
20-39	7264	39.1
40-49	2757	14.8
50-59	2024	10.9
60-69	2574	13.9
>69	3951	21.3
Gender	18570	100
Male	8705	46.9
Female	9865	53.1
Race/ethnicity	18570	100
Non-hispanic white	8096	43.6
Non-hispanic black	5013	27.0
Hispanic	4754	25.6
Other	707	3.8
Income (dollars)	18142	100
<$20,000	8993	49.6
>$20,000	9149	50.4
Marital status	18497	100
Married	11081	60.0
Widowed	2326	12.6
Divorced	2046	11.0
Never married	3026	16.4
Education	18371	100
8 years	4586	25.0
9-11 years	3057	16.6
12 years	5525	30.1
>12 years	5203	28.3

The age of the subject population was divided into 5 groups. The largest percentage of subjects was in the 20-39 year old group (39.1%). The mean age of subjects with a negative history of heart attack was 48.46 years old. The mean age of subjects with a positive history of heart attack was 69.65 years old. The gender of the population was divided all but equally between males and females. Males accounted for 46.9%, and females accounted for 53.1% of the subjects. Race was divided into 4 groups. Non-hispanic whites accounted for 43.6%, Non-hispanic blacks accounted for 27.0%, Hispanic subjects accounted for 25.6% and the last group, Other, accounted for 3.8%. Income status of the subjects was broken into two groups, >$20,000 (49.6%) and <$20,000 (50.4%). Marital status was divided into four groups. Married subjects were the largest group with 60.0% of the total. Those subjects never married were 16.4% of the population. Widowed and Divorced groups were similar at about 12% each. The amount of education of the subjects was divided into four groups. The first group is subjects who had 8 years of education (25.0%). The second group consists subjects who have had 9-11 years of education (16.6%). The third group consists subjects who have had 12 years of education (30.1%). The final group consisted of subjects with >12 years of education (28.3%).

Table 2. Cardiovascular risk factors for study population

Variable	N	%
Diabetes	18548	100
Yes	1580	8.5
No	16968	91.5
Hypertension (>140,>90)	18398	100
Yes	5321	28.9
No	13077	71.1
Cholesterol (mg/dL)	14750	100
<200	8952	60.7
>200	5798	39.3
Smoking	9570	100
Yes	4799	50.1
No	4753	49.9
Body mass index	14834	100
<25	4780	32.2
25-29	3718	25.1
>29	6336	42.7
C-reactive protein (mg/dl)	18570	100
<2.1	4730	30.4
2.1-5.0	11469	61.8
>5.0	2371	8.2

Table 2 shows several cardiovascular risk factors for the study population.   The

percentage of subjects with either Type I or Type II diabetes was 8.5%. This is consistent

with the national prevalence of 8.2%. The NHANES III categorized a patient as

hypertensive if a participant reported any of the following: patient was told that she or he

had high BP, current use of medications to treat high BP, or if the average measured BP

was $\geq$140 mmHg systolic, or $\geq$90 mmHg diastolic. 28.9% of subjects were classified as

hypertensive. The cutoff value for high or low cholesterol was 200mg/dL. 60.7% of the

subjects had normal cholesterol, while 39.3% have high cholesterol. Subjects who

currently smoke accounted for 50.1% of the subject population. Body mass index (BMI)

was calculated as weight in kilograms/height in meters squared, or weight (lbs) ÷ height

(in) ÷ height (in) × 703. BMI was divided into 3 groups: Health weight (<25),

Overweight (25-29) or Obese (>29). Healthy BMI subjects accounted for 32.2% of the

subject population. Overweight subjects accounted for 25.1% of the population. The

largest BMI group was the obese group, which accounted for 42.7% of the population.

C-reactive protein data is divided into 3 groups. At the time when the NHANES III CRP

level analysis was performed, high-sensitivity CRP analysis was not available. CRP

levels less than 2.1mg/dL were not quantifiable. With this less sensitive method, the

NHANES III defined the lowest level of CRP as <2.1mg/dL. The second level of CRP is

2.1-5.0mg/dL. The highest level of CRP is >5.0mg/dL. This study used data from the

NHANES III, which shows that 30.4% of the subjects had CRP levels less than

2.1mg/dL. The second CRP group in the NHANES III (2.1-5.0mg/dL) accounted for

61.8% of the subjects. The last CRP group (>5.0mg/dL) accounted for 8.2% of the

subjects. The CRP data shows that approximately 70% of the subjects had moderate to

severe elevation in CRP levels.

Variable	N	%
Dental examination	17809	100
Yes	16593	97.1
No	496	2.9
Dentate status	15847	100
Edentulous	1176	7.2
Teeth present	14671	92.8
Mean attachment loss	8901	100
<4mm	7352	82.6
>4mm	1549	17.4
Mean probing depth	8901	100
<3mm	6090	68.4
>3mm	2811	31.6
Gingival bleeding (% sites)	9954	100
<20%	2932	29.5
21-40%	541	5.4
>40%	6481	65.1

Table 3. Oral health variables of study population

Oral health data can be seen in Table 3. Of the 17,809 subjects responding to the

history of a dental exam question, 97.1% said they had undergone a dental exam in the

past. The dentate status of the subjects was also included. 92.8% of subjects reported

they had teeth present. Mean attachment loss data was divided into two groups, greater

than or less than 4 mm. 82.6% of subjects had <4mm of mean attachment loss, and

17.4% had attachment loss greater than 4 mm.   Mean Probing depth is given as either <3

mm or >3mm. 31.6% of the subjects had a mean probing depth >3mm. This finding

corresponds with the work of Oliver and Brown (1998), which showed that

approximately 30% of the population, has periodontitis. Subjects were divided into three

groups based upon bleeding on probing(BOP). 29.5% of the subjects had <20% of sites

with BOP. 5.4% of the subjects had between 20 and 40% of sites with BOP. The

greatest number of subjects (65%) had greater than 40% of sites with BOP. The BOP

results of this study are consistent with previous studies for a randomly selected

population.[52]

Table 4. Adjusted odds ratios for study population with positive history of heart attack

Variable	% with history of Heart attack	Odds Ratio	95%CI
C-reactive protein (mg/dL)			
<2.1	6.8		
2.1-5.0	76.8	2.81	[1.84,4.71]
>5.0	16.4	2.64	[1.59,5.11]
Diabetes			
No	77.6		
Yes	22.4	1.89	[1.29,2.77]
Hypertension (>140,>90)			
No	57.7		
Yes	42.3	1.82	[1.33,2.50]
Cholesterol (>,< 200 mg/dL)			
<200	54.0		
>200	46.0	1.69	[1.25,2.32]
Age (years)			
20-39	2.5		
40-49	14.8	12.08	[1.57,93.23]
50-59	10.8	22.68	[3.05,168.86]
60-69	23.8	39.82	[5.46,290.64]
>70	57.6	68.67	[9.41,501.27]
Gender			
Female	39.6		
Male	60.4	2.2	[1.53,3.18]
Race/ethnicity			
Non-hispanic white	63.4		
Non-hispanic black	19.5	1.75	[1.07,2.78]
Hispanic	14.6	1.82	[1.08,3.03]
Other	2.5	1.69	[0.57,6.25]
Marital status			
Married	61.2		
Widowed	25.9	2.07	[0.61,7.05]
Divorced	9.4	2.04	[0.57,7.26]
Never married	3.4	2.78	[0.76,10.14]
Education			
>12 years	19.7		
<9 years	37.2	1.28	[0.77,2.12]
9-11 years	19.6	1.69	[1.01,2.82]
12 years	23.5	1.53	[0.98,2.37]
Gingival Bleeding (% sites)			
<20%	29.0		
20-40%	6.7	1.95	[1.02,3.71]
>40%	64.3	1.23	[0.86,1.74]
Smoking			
No	49.9		
Yes	50.1	2.93	[2.14,3.15]

Table 4 shows the results of the multiple regression model using the listed variables. All subjects on this table had a positive history of heart attack. The first group in each category is considered the control for comparison by the other groups. The number of subjects with a positive history of heart attack was 937, approximately 5% of the total subjects. The number of subjects with a negative history of heart attack was 17,633. The mean CRP level of subjects with a positive history of heart attack was 3.12 mg/dL. The mean CRP level of subjects with a negative history of heart attack was 4.42 mg/dL. The listed odds ratio values refer to the odds of those subjects with positive history of heart attack also falling into the specific category of the variable in question. The odds ratio for Subjects with a positive history of heart attack to also have a CRP level of 2.1-5.0 mg/dL is 2.81 compared to those subjects with CRP levels <2.1 mg/dL. The odds ratio for subjects to fall into the highest group of CRP was 2.64. This data is consistent with previous research showing a trend for increased odds ratio for higher CRP levels in subjects with a history of heart attack. Subjects with positive history of heart attack had a 1.89 odds ratio for diabetes. This increase risk for diabetes to be found in subjects with a history of heart attack is consistent with previous research. Patients with a history of heart attack also had an odds ratio of 1.82 for hypertension (>140 systolic, >90 diastolic). The odds of subjects having high cholesterol (>200) was 1.69. The category of age has the highest odds ratios for any parameter studied. Subjects with a history of heart attack have a much higher odds of being in the older age groups. Subjects between the ages of 40-49 have an odds ratio of 12.08. This increases to 68.67 for those subjects >70 years old. There is an adjusted odds ratio of 2.2 for subjects to be

male. When the data is adjusted, heart attack subjects have an increased odds (1.69-1.82) of being non-white. Those subjects who were never married have an adjusted odds ratio of 2.78, higher than that of both widowed and divorced subjects. When the data is adjusted, education no longer has a clear correlation with odds of heart attack. The odds ratio for heart attack subjects to be smokers was 2.93. Of the oral health parameters, only gingival bleeding resulted in an unadjusted increased odds ratio (1.25 with a 95%CI of [0.84,1.87]). When adjusted for other study variables, the odds ratio increased to 1.93 with a 95% CI [1.02,3.71]. Probing depth and attachment loss were not included because they were not found to be significant.

Discussion

The association between periodontal disease and cardiovascular disease has been the topic of numerous studies. The direct link has yet to be found. The results of this study supports the hypothesis that periodontal disease may directly and/or indirectly facilitate atherosclerotic events.

Numerous studies of late have shown an association between increased severity of periodontal disease and increased risk of cardiovascular disease (CVD).[5,36,44,54] This study found the adjusted odds ratio for heart attack when compared to attachment loss (AL) was not significant. This contradiction in findings may be due to the disease status of the subjects. At a mean of >4mm attachment loss, the subjects in this study may not have had active disease. The problem when analyzing AL is than the value does not allow differentiation between current and past disease. Attachment loss was recorded as a mean value, < or > than 4 mm. The AAP defines periodontal disease according to attachment loss. Attachment loss of 1-2mm is classified as mild periodontitis, if the attachment loss is 3-4 mm the disease is classified as moderate periodontitis, and a loss of 5mm or more is classified as severe periodontitis. Noack et al.,[4] examined CRP and periodontal disease. In that study, attachment loss was divided into 3 groups. The first group was a control groups with ≤2 mm of attachment loss. The second groups was the mild group with >2 mm and ≤3 mm of attachment loss. The final group was the severe group with >3 mm of attachment loss. Noack also used high-sensetivity test for CRP.

Noack found that subjects with high levels of attachment loss had significantly higher CRP levels (4.06 mg/dL vs. 1.70 mg/dL for controls). Due to the attachment loss classification discrepancy, it is difficult to compare the current study with the Noack study. Previous studies have also shown a possible association between periodontal disease and atherosclerosis, myocardial infarct and stroke.[13,35,53] Arbes et al.,[36] found the odds ratio for heart attack increased with the severity of periodontal disease. After adjusting for age, sex, race, poverty, smoking, diabetes, high blood pressure, body mass index and serum cholesterol, subjects classified as mild periodontitis ($\leq$ 33% sites with $\geq$ 3mm attachment loss) had an OR of 1.4. Moderate periodontitis subjects (33-67% of sites with attachment loss $\geq$3mm) had an OR of 2.3. Severe periodontitis subjects (>67% of sites with attachment loss $\geq$ 3mm) had an OR of 3.8. In a study of subjects with a recent history of acute cardiovascular disease, Mattila et al.,[13] showed that patients with recent CVD had significantly more dental infections than healthy controls.

Past research comparing periodontal disease and CRP have shown that as the severity of periodontal disease increases so does the level of CRP.[4,5,6,54] Wu et al.,[54] found a significant relationship between indicators of poor periodontal health (bleeding, PD, AL) and increased CRP. The current study divided subjects into three groups based on the level of CRP. The lowest group included subjects with <2.1 mg/dL. The second group consisted of subjects in the range of 2.1-5.0 mg/dL. And the final group were those subjects with >5.0 mg/dL. A comparison of this study with others reveals a flaw in CRP level categories. As stated earlier, the NHANES III did not use hs-CRP to determine the level of CRP in the serum. 2.1 mg/dL was the lowest level of detectable serum CRP. This value is much higher than that shown to be within a healthy range.

Current literature supports that a CRP level of 2.1mg/dL is not healthy and is in the
medium-risk category for cardiovascular events.[2] Loos et al.,[5] found that healthy controls
had a median CRP value of 0.90 mg/dL, while generalized periodontitis subjects had a
CRP level of 1.45 mg/dL. Noack et al.,[4] reported the range for CRP as a risk factor for
CVD, peripheral vascular disease, or stroke is 1.34 mg/dL to 6.45 mg/dL with a mean of
3 mg/dL. Slade et al.,[55]and Koenig et al.,[56] found that CRP level of 3.0 mg/dL is the
mean value correlated with high risk for cardiovascular events. In the present study, the
mean CRP level of subjects with a positive history of heart attack was 3.12 mg/dL.
Ebersole et al.,[6] found that patients with chronic periodontitis had a mean CRP level of
9.12mg/dL compared to healthy controls who had a mean CRP level 2.17 mg/dL.

CRP is involved with the acute phase response which is associated with
inflammation and infection. An increase in CRP occurs with bacterial infections,
inflammatory conditions (acute rheumatic fever, acute rheumatoid arthritis), myocardial
infarction, transplant rejection, embolus, inflammatory bowel disease, during the last half
of pregnancy, with oral contraceptives, and with some malignancies.[17] Due to the fact
that periodontal disease is a chronic low-grade bacterial infection, activation of the acute
phase response and subsequent increase in CRP appears likely. The exact cause and
effect relationship is hard to verify due to the numerous separate factors which also cause
an increase in CRP. Ridker et al.,[57] examined the role of stain therapy on CRP levels.
Subjects with low lipid levels and high CRP were able to decrease their CRP level 14.8%
by taking a statin drug alone (lovastatin). Ebersole et al.,[6] administered a statin drug
(flurbiprofen) to patients with chronic periodontitis and was able to lower CRP levels by
35-40% without any other therapy. The body mass index of the subject will also affect

the level of CRP. The likely association with adipose and CRP levels involves cytokines. Human adipose tissue expresses and releases IL-6, a potent proinflammatory cytokine.[58] Visser et al.,[59] found that increased BMI value resulted in increased CRP levels. In that study, subjects classified as both overweight and obese were found to have increased levels of CRP. These authors state that the health of both overweight and obese subjects is similar to a person that has a low grade systemic inflammation. In the present study, when compared to BMI value, there was no significant increase in odds ratio for a heart attack.

Systemic health variables had odds ratios consistent with previous studies. Heart attack positive subjects were associated with increased odds ratio of elevated CRP levels 2.81(2.1-5.0 mg/dL) and 2.64 (>5.0 mg/dL). There was an increased odds ratio (1.89) for diabetes in subjects with history of heart attack. This is consistent with known risk. The American Heart Association[60] estimates that the prevalence of hypertension in diabetics is 1.5 – 3 times higher than nondiabetics and between 40-60% of subjects with diabetes have high blood pressure. Heart attack positive subjects in this study had an adjusted odds ratio for hypertension of 1.69. The fact that hypertension is associated with risk of heart attack is not surprising. Blood pressure is believed to be a critical factor for cardiovascular health. Increased blood pressure increases the risk of cardiovascular disease.[61] In a meta-analysis of over 400,000 subjects, a 7–mm Hg increase in diastolic blood pressure over any baseline reading was associated with a 27 percent increase in CHD risk and a 42 percent increase in stroke risk.[62] In this study, subjects with a positive history of heart attack had and odds ratio of 1.69 to also have high cholesterol level (above 200 mg/dL). Periodontal disease has been linked to higher levels of cholesterol.

Losche et al.,[63] found there was significantly higher levels of total cholesterol, LDL,

triglycerides and blood glucose in periodontally diseased subjects. Numerous

retrospective and cross-sectional studies suggest the association between increased LDL

and vascular risk.[29] Medical treatment of high cholesterol generally focuses on diet,

exercise and statin drugs such as atorvastatin (Lipitor). These statins are competitive

inhibitors of HMG-CoA reductase, a rate-limiting enzyme of cholesterol synthesis.

Ridker et al.,[64] found the combination of TC:HDLC had a relative risk of future

myocardial infarction to be 3.0, among apparently healthy middle aged men. Statins have

been shown to decrease risk of coronary events in patients with high lipid levels

regardless of the CRP levels, due to their effect on lipid profiles.[57] Age had the highest

odds ratio for association with positive history of heart attack. Subjects >70 years old

had an odds ratio of 68.67. Male subjects had an adjusted odds ratio of 2.2 for an

association with positive history of heart attack. There was an adjusted odds ratio of

1.69-1.75 for heart attack subjects to be non-white. Increased odds ratio for heart attack

subjects to be non-married (O.R. 2.04 - 2.78). The 95% confidence interval (<1) of the

marriage data brings into question the actual significance of this variable. After adjusting

for confounding variables, the education level of the subjects was not significant at 95%

CI. Those subject who smoked (50.1% of the sample population) had an adjusted odds

ratio for an association with positive history of heart attack of 2.93. The center for

disease control (CDC) reported 26.4% of adult men and 22.0% of adult women in the

United States are smokers, representing 47.2 million lives.[65] There is a

disproportionately high number of smokers (50.1%) in the present study. This may result

in higher odds ratio for an association with heart attack. Regardless, smoking is a

significant risk factor for heart disease, and the subjects with positive history of heart attack had a higher odds ratio association with smoking. The CDC also reported that smoking had a relative risk of 2.5 for coronary heart disease. Overall, smokers have a 70% greater level of CVD risk than nonsmokers and persons who smoke at least 2 packs of cigarettes per day have a two- to threefold greater risk for CVD.[65]

The Interesting finding of this study involves the association between gingival bleeding and heart attack. Bleeding was the only significant oral health factor associated with increased risk of heart attack. Neither probing depth, nor attachment loss was found to be significant. The adjusted odds ratio for heart attack positive subjects to be within the 20-40% BOP group was 1.95. The adjusted odds ratio for subjects with >40% of sites with bleeding was 1.23. As discussed earlier, bleeding is a sign of inflammation and when it is used collectively with other clinical findings, bleeding can be a predictor of future disease progression. The results of this study agree with that of Buhlin et al.[44] In that study, bleeding was the only significant factor for increased odds ratio for self reported heart attack. Buhlin used questionnaires of 2,839 randomly selected Swedish subjects. The questionnaire asked knowledge about dental care habits, oral health, cardiovascular disease and socioeconomic variables of income, education etc. Buhlin found there was a significant association between self-reported bleeding gums (odds ratio 1.60) and known cardiovascular disease. This current study also agrees with the finding of Morrison et al.[66] In that retrospective cohort study, using data from the 1970-1972 Nutrition Canada Survey (NCS), Morrison found that subjects with mild or severe gingivitis had a higher relative risk (RR 2.15, 95%CI) of dying of coronary heart disease than those subjects with periodontitis. Bleeding may be more accurate than the

measurement of attachment loss or probing depths when assessing active periodontal disease. This study supports the association of oral health findings as markers for risk of cardiovascular disease.

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